Protein-Ligand Interactions II

3062-Pos Board B109

Mechanism of Interaction between the General Anesthetic Halothane and a Model Ion Channel Protein: Structural Investigations via X-ray Reflectivity from Langmuir Monolayers

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De novo designed model membrane proteins provide an alternate platform for investigating the interaction of general anesthetics and proteins, particularly for obtaining structural information, while avoiding the complexity of natural membranes. We previously reported the synthesis and structural characterization of a model membrane protein comprised of an amphiphilic 4-helix bundle peptide with a hydrophobic domain based on a synthetic ion channel and a hydrophilic domain with designed cavities for binding the general anesthetic halothane (Ye *et al,* Biophys. J. **87:** 4065 (2004)). In the present work we synthesized an improved version of this halothane-binding amphiphilic peptide with only a single cavity and an otherwise identical control peptide with no such cavity, and applied x-ray reflectivity to monolayers of these peptides in order to probe the distribution of halothane along the length of the core of the 4-helix bundle as a function of the concentration of halothane. At the lower concentrations achieved in this study, about three molecules of halothane were found to be localized within a broad symmetric unimodal distribution centered about the designed cavity. At higher concentrations, about six-seven molecules were found to be uniformly distributed along the length of the bundle, corresponding to approximately one molecule per heptad. Monolayers of the control peptide showed only the latter behavior, namely a uniform distribution along the length of the bundle irrespective of the halothane concentration. The results provide insight into the nature of such weak binding when the dissociation constant is in the mM regime, relevant for clinical applications of anesthesia. They also demonstrate the suitability of both the model system and the experimental technique for additional work on the mechanism of general anesthesia. Supported by NIH GM55876.

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Probing Peptide Material Interactions At The Single Molecule Level

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Optical tweezers are well suited to probe the characteristics of molecular scale interactions at the interface between biology and materials. The high resolution force and position measurements can offer unique insights into the mechanism and kinetics of adhesion between short peptide sequences, aptamers, and a variety of material surfaces used in microfluidics, medicine, and semiconductor technologies. We have developed a flexible assay to characterize the binding distribution and off-rate for a collection of peptides. The peptide sequences of interest are attached to a polystyrene bead via a 3500 bp DNA spacer using a cysteine on the peptide and a primary amine on the DNA. The modularity of the assay allows any peptide sequence containing a N-terminal cysteine to be linked to the DNA and evaluated. Casein and on certain surfaces, the DNA, act as blocking molecules to reduce non-specific adhesion. Two previously elucidated peptide sequences selected for sapphire affinity were examined and exhibited dissimilar binding distributions, mean adhesion force, and rate constants. To explore the broad applicability of the assay, the adhesion of a universal binder on slide glass was inspected.

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Effects of Load and Contact Time on the Stability of Bimolecular Integrin-Fibrinogen Bonds Under a Constant Tensile Force

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The regulated ability of integrin alphaIIbbeta3 to bind fibrinogen plays a crucial role in platelet function, thrombosis, and hemostasis. Previously, we found that the rupture force distribution of individual integrin alphaIIbbeta3-fibrinogen interactions under ramp loads display at least two components that differ in kinetics, loading rate dependence, and susceptibility to activation/inhibition of the integrin, which suggests that specific binding and unbinding of the integrin alphaIIbbeta3 with fibrinogen is a complex multi-step process (Litvinov et al., Biophys J. 2005; 89:2824). Employing a new laser tweezers-based electronic force clamp (Litvinov et al., Biophys J. 2008, 94, Suppl., Abstract, 1724-Pos.), we now study interactions of purified integrin alphaIIbbeta3 and fibrinogen under constant tensile force, mimicking the effect of hydrodynamic blood flow on an adherent platelet. Under a constant tensile force of 50 pN, the distribution of durations of the alphaIIbbeta3 and fibrinogen bonds is bimodal, with specific integrin-fibrinogen interactions mostly lasting more than 2 sec. At the same constant unbinding force, the bond lifetimes increase as the duration of contact between alphaIIbbeta3- and fibrinogen-coated interacting surfaces is increased from 0.1s to 2s, again suggesting that the initial interaction of fibrinogen with alphaIIbbeta3 is followed by reorganization of the binding interface, enhancing the strength and stability of binding. In further experiments, the average bond lifetimes exponentially decrease as tensile force is increased from 0 to 30 pN, suggesting that in this force range the alphaIIbbeta3-fibrinogen interactions represent classical ''slip bonds''. Taken together, these data provide important quantitative characteristics of the alphaIIbbeta3-fibrinogen binding and unbinding, which underlie the dynamics of platelet adhesion and aggregation in the blood flow.

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Solvent Exposure- and Distance-Dependent Dielectric Function for Ligand-Protein Interactions

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Physics-based force fields for high throughput ligand docking usually determine electrostatic energy with distance-dependent dielectric (DDD) functions, which do not fully account for the dielectric permittivity variance between ~ 2 in the protein core and ~ 80 at the protein surface. We propose here a new dielectric function that depends on the distance between a pair of interacting atoms and the degree of their exposure to the aqueous environment. This solvent exposure- and distance-depend dielectric (SEDDD) function accounts for both electrostatic and dehydration energy components. Using a training set of ten x-ray structures, we first optimized the global-minimization protocol in the ZMM program with the AMBER force field, implicit solvation, and the DDD function $\epsilon = 2r$. A search was considered a success if the root mean square deviation (RMSD) of the ligand's atoms in the apparent global minimum from the x-ray structure was less than 2 Å. For each complex, the apparent global minimum was found by sampling hundreds of thousands of the rigid-ligand binding poses and refining low-energy poses by Monte Carlo-minimizing energy with flexible ligand and flexible protein. For an examining set of 60 structures, the global-minimization protocol with implicit solvation and various DDD functions ($\epsilon = r$, $\epsilon = 2r$, and $\epsilon = 4r$) yielded success rates of 66.7%, 73.3% and 75.0% respectively. In most outliers, the ligand-binding sites were located at the protein surface. Using a second training set of 16 ligand-protein complexes, we parameterized the SEDDD function to minimize the average RMSD between the apparent global minima and x-ray structures. Application of the parametrized SEDDD function to the examining set yielded a success rate of 91.7%, a substantial improvement versus the best-performing DDD function with implicit solvation.

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IRRAS Studies of the Host Defense Effect of Pulmonary Surfactants SP-A and SP-D

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The pulmonary surfactant proteins SP-A and SP-D have been proven to play a role in host defense. SP-A binds phosphatidylcholine, galactosylceramide, and the lipid A portion of lipopolysaccharide (LPS), which is found in Gram-negative bacterial cell walls. SP-D binds phosphatidylinositol, glucosylceramide and LPS. Although X-ray crystallography and NMR spectroscopy have provided atomic level information about SP-A and SP-D structures, molecular level information about their binding to biological ligands is lacking. IR reflection-absorption spectroscopy (IRRAS) is used in the current work to study the properties of SP-A and SP-D at air/water interfaces and their interaction with biological ligands. The monolayer properties of recombinant rat neck + carbohydrate recognition domain (NCRD) SP-A and its point mutant D215A SP-A are compared. Both NCRD SP-A and D215A SP-A adsorb to the air/water interface, DPPC monolayers, and Lipid A monolayers, but D215A/PL SP-A displays higher affinity and greater stability. Measurements of surface pressure, Amide 1 intensity, and lipid acyl chain conformational ordering can help to elucidate the mechanism of interaction of SP-A and its ligands. Similar experiments have been carried out with SP-D.

3067-Pos Board B114

Communication Via Structural Water: Changes In The Thrombin Water Channel And Active Site Due To Sodium Binding

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Thrombin plays a pivotal role in the blood coagulation cascade, functioning as both a procoagulant and anticoagulant. Thrombin exists in the blood in equilibrium between these two catalytic forms, and the transition from the slow to the

fast form is initiated by sodium binding. Allosteric communication between the sodium binding site and active site is necessary for substrate recognition and enzyme function: specifically, the conversion from the slow form to the fast form upon Na^+ binding. Thrombin contains a water channel that extends from the Na^+-binding site to the active site. It has been suggested previously that this water channel may play a role in the allosteric communication between the Na^+-binding site and the active site. We have analyzed water channel structure and fluctuations in *apo* and *holo* (Na^+-bound) forms of thrombin using molecular dynamics. Our results show that the water channel of thrombin exists in three distinct states. States of large channel volume and high water occupancy are observed more often when the Na^+-binding site is empty. These large volume/high occupancy states include a water channel that completely connects the Na^+-binding site to the active site through a network of hydrogen bonds. Furthermore, the large volume/high occupancy water channel causes significant structural changes in the active site, including disruption of hydrogen bonding in the catalytic triad. Conversely, when the cation binding site is occupied by a Na^+-ion, the water channel has a significantly smaller volume, fewer water molecules, and does not provide a complete hydrogen bond network between the Na^+-binding site and the active site. We analyze these results in the context of changes in the catalytic activity of thrombin in the presence and absence of Na^+.

3068-Pos Board B115

The Importance of Electrostatic Effects in the Recognition of NPF Motifs by the EH Domains of Mammalian EHD Proteins

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EHD proteins are membrane remodeling proteins that control exit of receptors from the recycling endosome and their return to the plasma membrane. They consist of a nucleotide binding domain and an EH domain, which is a small Ca^{2+} binding module composed of 2 EF hands. EH domains bind to asparagine-proline-phenylalanine (NPF) motifs, which are found in various proteins, often in multiple copies, throughout the vesicle trafficking pathway. The mechanism by which EH domains discriminate between individual NPF containing proteins is not well understood. There are 4 closely related EHD proteins in mammals, termed EHD1-4. We have measured the affinities the EH domains from all 4 EHD proteins for 11-residue NPF containing peptides derived from Rabenosyn-5 and Rab11-Fip2 using isothermal titration calorimetry. The highly acidic Rabenosyn peptides bind with affinities of the order of $6*10^5\ M^{-1}$; this is an order of magnitude higher than was found for the neutral Rab11-Fip2 peptide. The Rabenosyn peptide interaction was also found to be salt dependent. In a second set of experiments, we synthesized EH domains from EHD1 and EHD4 with short NPF containing sequences from derived from Rabenosyn or Rab11Fip2 tethered to the C-terminus. Fluorescence quenching experiments showed that a tryptophan residue at the base of the NPF binding pocket was protected by the tethered NPF. All tethered NPF sequences enhance the stability of the EH domain to urea denaturation, but the Rabenosyn sequence (13 $kJmol^{-1}$) is more effective than the Rab11-Fip2 sequence (9 $kJmol^{-1}$). Our results suggest that negative charges directly following the NPF sequence strongly enhance the affinity of EHD EH domains for their target sequences.

3069-Pos Board B116

Analyzing Electrostatic Determinants of Affinity and Promiscuity in the HIV-1 Reverse Transcriptase System Using Charge Optimization

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Electrostatic charge optimization (Lee and Tidor, *J. Chem. Phys.* 1997) was used to study the binding site of wild type and mutant HIV-1 reverse transcriptase (RT) complexed with the non-nucleoside inhibitors nevirapine and rilpivirine (TMC-278). Our ultimate goal is to analyze and to further understand the electrostatic determinants of tight binding and broad molecular recognition toward this rapidly-mutating target. For each inhibitor-RT mutant pair, we computed the drug's optimal charge distribution - the hypothetical charge distribution that would bind to the target variant more tightly than any other isosteric drug. By comparing these optimal charge distributions with each drug's actual charge distribution, we are able to identify potential sites of electrostatic non-complementarity that may be altered to increase binding affinity. Additionally, by comparing the electrostatic optima for a given drug toward multiple RT variants, we can gain insight into the sensitivity of the electrostatic determinants of binding to the variability in RT as a result of certain drug resistance mutations.

3070-Pos Board B117

A Thermodynamic Study Of Ligand Access/escape From Protein Cavities

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Insulin, a protein hormone, regulates glucose homeostasis and carbohydrate metabolism in higher organisms. Therapeutic formulations of hormone are preserved against degradation and denaturation by antimicrobial preservatives such as phenols, cooperative binding of which stabilizes hexameric complexes of insulin. Dissociation of hexameric species (on minutes to days time scale) into biologically active monomers is facilitated by rapid unbinding of phenols (on milliseconds time scale). However, a clear understanding of dissolution kinetics and determinants of the rates of ligand unbinding remains obscure, chiefly due to unresolved ambiguities in NMR results. We have used random acceleration molecular dynamics (RAMD) to identify and characterize a variety of potential ligand dissociation mechanisms. We observe three distinct exit routes for the ligand and resolve potentials of mean force (PMFs) along them by performing free energy calculations. Free energy profiles for each mechanism are computed with the help of second order cumulant expansion of Jarzynski's equality and non-equilibrium work statistics gathered from multiple independent steered molecular dynamics (SMD) simulations. Based on energetic barriers and structural properties, we suggest a plausible preferred mechanism for the ligand exchange. The most likely pathway with the lowest free energy barrier involves a leap over the ''gate'' formed by HisF5 and IleA10, with simultaneous passage of the ligand through a narrow channel existing between LeuA13, LeuH17, and the ''gate''. Free energy profiles also display several weakly-bound metastable states for the ligand during entry and exit from R6 insulin hexamer.

3071-Pos Board B118

Expression of, and Preliminary Biophysical Characterization of a Minimal Binding Domain peptide of TSC2 that binds to the small GTPase Rheb

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Rheb (Ras homolog enriched in brain) is a recently identified Ras protein the cycles between GTP-and GDP-bound states similarly to other Ras proteins. An important interaction between Rheb and the Tuberous Sclerosis complex proteins (TSC1/2) mediates the mammalian Target Of Rapamycin (mTOR) pathway, which regulates cell growth, energy and nutrient levels. TSC2 contains a GTPase-binding domain near its C-terminus that contains a highly conserved 15-amino acid region. Mutations in this region has resulted in altered GTPase activity towards Rheb, suggesting that the 15 amino acid conserved region is vital for this interaction.

To date, molecular details of this important Ras-effector interaction are unknown. We present here a preliminary characterization of an interaction of Rheb with a minimal GTPase-binding domain peptide derivative of TSC2. A series of peptides truncated from the C-terminal region of TSC2 encompassing the GTPase-binding domain were expressed in bacterial cell lines. A peptide of 37 amino acids (TSC2-37) has demonstrated similar binding affinity to Rheb in-vitro to a 900 amino acid C-terminal derivative of TSC2. Isothermal titration calorimetry was used to examine the binding affinity of a synthetic peptide consisting of only the15 amino acids of the conserved GTPase-binding region of TSC2 for Rheb to highlight the importance of the conserved region in binding. These results represent a first step towards the biophysical characterization of the Rheb-TSC2 complex that is expected to provide molecular details that can be translated to its biological function.

3072-Pos Board B119

Biochemistry on a Leash: Confinement as a Regulatory Mechanism for Bimolecular Reaction Rates

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We describe two mechanisms by which confinement is utilized to regulate diffusion-limited bimolecular reaction rates. The first mechanism, illustrated by the actin capping protein formin, uses a flexible polymer to tether ligand binding sites, which serve as intermediaries, to the reactive site. The second mechanism uses a potential (e.g. hard wall potential), to constrain the motion of a ligand receptor within a confining volume. We analyze both mechanisms theoretically, using a combination of analytic and numerical techniques, to obtain the steady state binding kinetics. We explore how the reaction rates are regulated by parameters of the model such as the length of the polymer tether, and use our findings to explain the key features of the formin system. Finally, we suggest other systems, both synthetic and biological, in which these mechanisms for regulating bimolecular reactions might be at play.

3073-Pos Board B120

A Comparative Study On The Interaction Of New Designed Aliphatic Pd(II) Complexes With Human Serum Albumin

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